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Tyrosinemia Type 1 and symptoms of ADHD: Biochemical mechanisms and implications for treatment and prognosis

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Hereditary tyrosinemia Type 1 (HT-1) is a rare metabolic disease where the enzyme catalyzing the final step of tyrosine breakdown is defect, leading to accumulation of toxic metabolites. Nitisinone inhibits the degradation of tyrosine and thereby the production of harmful metabolites, however, the concentration of tyrosine also increases. We investigated the relationship between plasma tyrosine concentrations and cognitive functions and how tyrosine levels affected enzyme activities of human tyrosine hydroxylase (TH) and tryptophan hydroxylase 2 (TPH2). Eight Norwegian children between 6 and 18 years with HT-1 were assessed using questionnaires measuring Attention Deficit Hyperactivity Disorder (ADHD)-symptoms and executive functioning. Recent and past levels of tyrosine were measured and the enzyme activities of TH and TPH2 were studied at conditions replicating normal and pathological tyrosine concentrations. We observed a significant positive correlation between mean tyrosine levels and inattention symptoms. While TH exhibited prominent substrate inhibition kinetics, TPH2 activity also decreased at elevated tyrosine levels. Inhibition of both enzymes may impair syntheses of dopamine, noradrenaline, and serotonin in brain tissue. Inattention in treated HT-1 patients may be related to decreased production of these monoamines. Our results support recommendations of strict guidelines on plasma tyrosine levels in HT-1. ADHD-related deficits, particularly inattention, should be monitored in HT-1 patients to determine whether intervention is necessary.

KEYWORDS
ADHD, dopamine, hereditary tyrosinemia Type 1, inattention, serotonin

1 | INTRODUCTION

Many metabolic diseases influence brain function and are associated with psychiatric symptoms and neuropsychiatric disorders (including autism-spectrum disorders, ADHD and psychotic disorders). ADHD is a common neurodevelopmental disorder with symptoms of either hyperactivity/impulsivity, or inattention, or both (American Psychiatric Association, 2013). ADHD has high rates of comorbidity with psychiatric or somatic disorders, possibly reflecting shared pathophysiological mechanisms (Instanes, Klungsoyr, Halmoy, Fasmer, & Haavik, 2018). Knowledge about the relationship between neurometabolic disorders (NMDs) and symptoms of ADHD may provide insight into the etiology of ADHD, as well as improve the clinical management of patients with such conditions. As symptoms of ADHD have been

linked to low dopamine levels in prefrontal cortex, studies of metabolic disorders influencing the dopamine system have been of particular interest. Similarities in neurodevelopmental functioning have been found, for example, between treated phenylketonuria and ADHD (Stevenson & McNaughton, 2013), but also between ADHD and hereditary tyrosinemia Type 1 (HT-1; OMIM 276700; Pohorecka et al., 2012).

Hereditary tyrosinemia Type 1 is an autosomal recessive disease caused by loss-of-function mutations in the gene encoding fumarylacetoacetate hydrolase (FAH; EC 3.7.1.2), the last enzyme in the tyrosine degradation pathway. The incidence in most of the world is estimated to be 1 in 100–120,000 live births. In Norway, incidence is approx. 1 in 74,800 live births (Bliksrud, Brodtkorb, Backe, Woldseth, & Rootwelt, 2012). Lack of functional FAH leads to accumulation of metabolites like fumarylacetoacetate and succinylacetone, which causes organ damage, including progressive liver disease with pronounced cirrhosis, regeneration and secondary renal tubular dysfunction. Individuals with the most acute form present with severe liver failure within weeks after birth, whereas patients with the chronic form may present with hypophosphatemic rickets, cirrhosis, and hepatocellular carcinoma (HCC; De Baulny, 2014; Trahms, 2001). Untreated, these patients die from cirrhosis or HCC at a young age.

Following the diagnosis, individuals with HT-1 are treated with the drug nitisinone, combined with a protein-restricted diet. Nitisinone (2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC)) reversibly inhibits the degradation of tyrosine at an earlier step by acting on 4-hydroxyphenylpyruvate dioxygenase (HPPD), thus protecting the liver against the carcinogenic metabolites, but the tyrosine level remains elevated. Nitisinone has dramatically improved the prognosis of HT-1 (G. Mitchell, 2015), especially if treatment is started early, but it is unclear if the phenylalanine and tyrosine-reduced diet is sufficient to reduce plasma tyrosine to levels that prevent cognitive deficits (Bendadi et al., 2014). In patients with tyrosinemia Type III with similar concentrations of tyrosine as treated HT-1 patients, and in tyrosinemia Type II, with even higher levels of tyrosine, neurocognitive difficulties are prominent (G. A. Mitchell, Grompe, Lambert, & Tanguay, 2001; Natt, Kida, Odievre, Di Rocco, & Scherer, 1992). This is in accordance with studies showing learning difficulties (Masurel-Paulet et al., 2008), lower IQ (Thimm et al., 2012), suboptimal motor function (Thimm et al., 2012), difficulties with social cognition (van Ginkel et al., 2016), inattentiveness (Pohorecka et al., 2012), and difficulties with working memory (van Ginkel et al., 2016) among treated HT-1 patients. See Figure 1 for information about the catabolic pathways for the different types of tyrosinemia and nitisinone.

Several mechanisms have been suggested for the neurodevelopmental problems observed in HT-1, including direct toxic effect of high tyrosine, negative sequelae of severe liver disease before treatment, impaired influx of amino acids into the brain, decreased serotonin in the central nervous system, low levels of phenylalanine in blood and direct negative effects of nitisione (van Ginkel, Jahja, Huijbregts, & van Spronsen, 2017). In addition, a possible relationship between high tyrosine levels and high dopamine has been

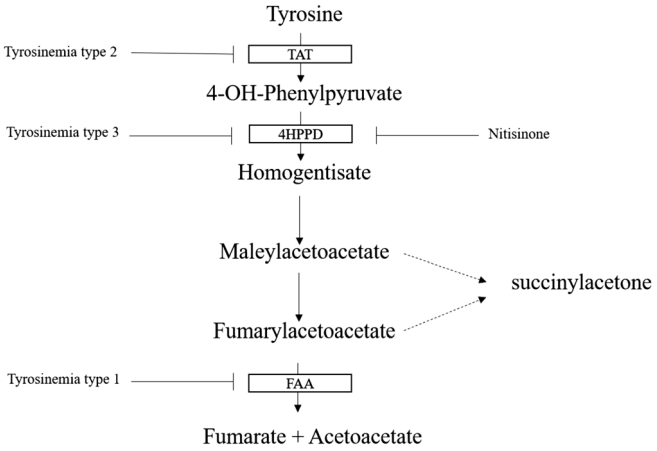

FIGURE 1 The tyrosine degradation pathway. The first, second and last steps are inhibited in tyrosinemia type 2, 3 and 1, respectively. In tyrosinemia type 1, fumarylacetoacetate and maleylacetoacetate are metabolised further to succinylacetone. Nitisinone is inhibiting 4HPPD, protecting against toxic metabolites. TAT, tyrosine aminotransferase; 4HPPD, 4-hydroxyphenylpyruvate dioxygenase; FAA, fumarylacetoacetase

suggested, as tyrosine is a precursor for dopamine (van Ginkel et al., 2017). Walker, Pitkanen, Rahman, and Barrington (2018) reported underperformance on neurocognitive tests in three patients with HT-1, with highest plasma tyrosine levels in the patient struggling most on the neurocognitive tests.

ADHD-symptoms may be related to decreased function of the catecholamine transmitters dopamine and noradrenaline in prefrontal cortex (Borodovitsyna, Flamini, & Chandler, 2017; Volkow et al., 2009). Biosynthesis of catecholamines relies partly on phenylalanine that is converted into tyrosine by the liver enzyme phenylalanine hydroxylase (PAH, EC 1.14.16.1). Thus, in classical PKU, mutations in the PAH gene diminish PAH activity, resulting in low levels of tyrosine, which leads to decreased dopamine levels in PKU (Antshel & Waisbren, 2003a). Although dietary treatment could prevent severe cognitive impairment, residual symptoms have been reported (Stevenson & McNaughton, 2013). This is supported by a study showing that 26% of children with treated PKU used central stimulants for attentional dysfunction, compared to 6.5% in a group with Type 1 diabetes mellitus (Arnold, Vladutiu, Orlowski, Blakely, & DeLuca, 2004). van Ginkel et al. (2017) have argued that nitisinone-treated HT-1 and PKU display similar neurodevelopmental characteristics as well. However, the proposed explanations for those features have been strikingly different, as treated HT-1 has been suggested to feature elevated dopamine levels in the prefrontal cortex. Understanding the relationship between HT-1 with ADHD and PKU could bring new insights into the underlying biological mechanisms behind HT-1, carrying the potential for improved treatment for the cognitive difficulties observed in this group.

Transport of large neutral amino acids across the blood–brain barrier (BBB) is mediated by endothelial L-type amino acid transporter 1 (LAT1, SLC7A5) in a heterodimeric complex with heavy chain 4F2 antigen (Yan, Zhao, Lei, & Zhou, 2019). The transport of amino acids

like leucine, isoleucine, phenylalanine, tyrosine, tryptophan, and methionine is therefore mediated by the same transporter system into or out of the brain, and is hence mutually competitive, depending on their affinity for LAT1 and their concentration in plasma or brain tissue, respectively (de Groot et al., 2013). Hyperaminoacidemias such as PKU and HT-1 are expected to affect the transport flux of amino acids into the brain. Thus, high circulating levels of phenylalanine in PKU will compete out other LAT1 transported amino acids such as tyrosine and tryptophan, lowering brain protein synthesis, as well as compromising biosynthesis of catecholamines and serotonin. This may lead to cognitive deficits (Yano, Moseley, Fu, & Azen, 2016). For nitisinone treated HT-1, high plasma levels of tyrosine, is expected to decrease the flux of other LAT1 transported amino acids into the brain—such as tryptophan—which may affect serotonin synthesis. Conventionally, the high levels of tyrosine have been suspected to increase catecholamine synthesis in the brain (van Ginkel et al., 2017).

Here we present a new hypothesis for the cognitive difficulties observed in HT-1, arguing that high levels of tyrosine may impair the synthesis of dopamine and norepinephrine, due to the pronounced substrate inhibition kinetics of tyrosine hydroxylase (TH, EC 1.14.16.2), the rate-limiting enzyme of catecholamine synthesis.

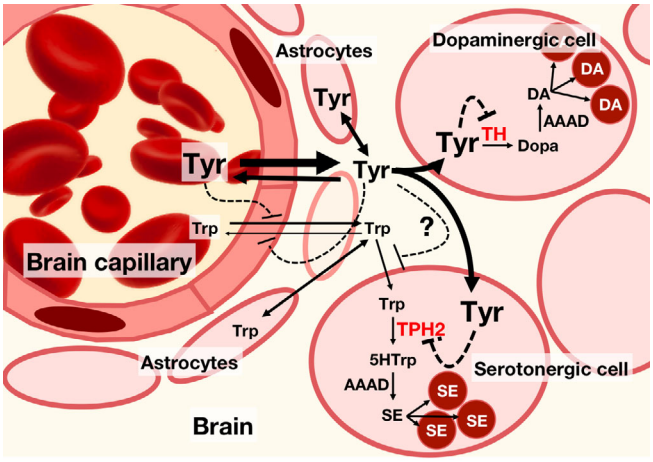

FIGURE 2 Tyrosine and tryptophan transport through the blood–brain barrier. The figure illustrates the impact of high plasma levels of tyrosine (Tyr) on its own transport into the brain and into target cells, as well as on transport of tryptophan (Trp). Transport of large, uncharged amino acids across the tightly junctioned endothelial cells of brain capillaries, a feature of the blood–brain barrier, is mediated by the L-type amino acid transporter via a mutual competitive kinetic mechanism. High Tyr levels will inhibit transport of Trp into and out of the brain. Inhibition of Trp transport into brain cells, for example, serotonergic cells by extreme high Tyr levels is also predicted. 3,4-dihydroxyphenylalanine (L-Dopa) is synthesized from Tyr by TH and further decarboxylated by aromatic amino acid decarboxylase (AAAD) to dopamine (DA). DA is subsequently transported and stored in synaptic vesicles. A similar pathway is used to synthesize serotonin (SE) from Trp, only that the first enzyme, tryptophan hydroxylase 2 (TPH2), mediates the hydroxylation of Trp to 5-hydroxytryptophan (5HTrp). The possible inhibition of TH and TPH2 by high levels of Tyr were investigated in this study

Dopamine is synthesized from tyrosine by TH and aromatic amino acid decarboxylase (AAAD, EC 4.1.1.26) prior to vesicular transport and storage (Figure 2), whereas norepinephrine is synthesized from dopamine by dopamine β-hydroxylase within these synaptic vesicles. TH hydroxylates tyrosine in an iron(II) dependent reaction, using the cosubstrate tetrahydrobiopterin (BH_4) and molecular oxygen. TH shows substrate inhibition kinetics for tyrosine even at physiological concentrations (Kumer & Vrana, 1996; Quinsey, Luong, & Dickson, 1998). Under normal conditions, substrate inhibition can be beneficial, as it has been suggested to stabilize the synthesis of catecholamines against fluctuations caused by variable dietary intake of tyrosine (Reed, Lieb, & Nijhout, 2010). An implication of this kinetic feature of TH is that the high levels of tyrosine in HT-1 may lead to substantial inhibition of the enzyme and thus a decrease of dopamine synthesis. We therefore hypothesized that both high and low levels of tyrosine may lead to impaired synthesis of catecholamines, which could explain the similarities found between PKU and HT-1.

The homologues enzyme of TH and PAH, tryptophan hydroxylase, catalyzes the first and rate-limiting step of serotonin synthesis (Figure 2). Low serotonin levels have also been reported in HT-1 (Thimm et al., 2011). In addition to the expected inhibitory effect of tyrosine on tryptophan transport into the brain, we hypothesized that high levels of tyrosine could also inhibit the activity of human tryptophan hydroxylase 2 (TPH2, EC 1.14.16.4), the rate-limiting enzyme of brain serotonin synthesis.

On this background, we aimed to measure core symptoms of ADHD in HT-1 and relate this to plasma levels of tyrosine. Especially inattention problems have been frequently reported by parents of children with treated PKU (using the ADHD RS-IV; Mooney, Prasad, & Shaffer, 2013), and a correlation between inattention scores and levels of phenylalanine in serum has been found in tetrahydrobioptein (sapropterin) responders (Wyrwich et al., 2015). Because of the proposed similarities between PKU and HT-1, inattention was also the main focus of this study. However, Barkley (2003) pointed out that although ADHD has been viewed as a disorder of primarily inattention and hyperactive–impulsive behavior, newer theories characterized deficits in executive functioning as essential to the disorder. Executive function problems are found to be associated with high levels of phenylalanine (Bilder et al., 2016). Because of the possible shared cognitive/behavioral phenotypes in PKU and ADHD, executive functioning is therefore also of particular interest when investigating HT-1.

In this study, we investigated the relationship between elevated plasma levels of tyrosine found in treated HT-1 patients and core symptoms of ADHD, namely, inattention, hyperactivity, and executive functioning deficits. We also assessed the effect of physiological and pathophysiological levels of tyrosine on the in vitro activity of human TH1, the major human TH isoform, as well as human TPH2, expressed in brain serotonergic neurons.

We asked the following questions:

1. Are ADHD-symptoms overrepresented in HT-1 patients in Norway?

2. Is the concentration of tyrosine in blood directly linked with the severity of ADHD-related symptoms and executive function performance in HT-1?
3. If so, could this association be explained by inhibition of TH and caused by elevated tyrosine levels?

2 | METHODS

2.1 | Sample description

All parents to children (0–18 years) with HT-1 in Norway were given oral and written information about the project. If children were 12 years or older, they also signed the informed consent form in addition to their parents. Project approval was granted by the Regional Committee for Medical Research Ethics of Western Norway (IRB 00001872). Eleven out of 12 eligible children initially participated in the project. However, to ensure sufficient sample homogeneity, only children between 6 and 18 years (N = 10) were included in the actual analyses. Two potential participants (2 and 3 years old) were excluded because of their young age and one participant with epilepsy was excluded, as epilepsy may cause cognitive difficulties in itself that are not directly related to HT-1 (although it may be possible that the epilepsy developed secondarily to HT-1 or its treatment). Diagnoses had been given biochemically with detection of the pathognomonic succinylacetone and confirmed using DNA sequencing with detection of known disease mutations.

2.2 | Instruments

Questionnaires were filled in by parents to rate symptoms of ADHD and executive functioning. Levels of tyrosine from 2009 to 2016 and during 2017 were calculated separately. This was done to study the mean level from the year questionnaires were filled in (2017), in addition to long-term levels. Effects of increasing tyrosine concentrations on TH and TPH2 were studied in in vitro experiments.

Parent forms of ADHD Rating Scale-IV (ADHD RS-IV; DuPaul et al., 1998) and behavior rating inventory of executive functioning (BRIEF; Gioia, Isquith, Guy, & Kenworthy, 2000) were used to measure inattention, hyperactivity, and executive functioning.

ADHD RS-IV is an 18-item rating scale that assesses ADHD-symptoms in children on a four-point Likert scale (0 = never or rarely, 1 = sometimes, 2 = often, 3 = very often). The instrument is divided into two subscales (hyperactive/impulsive and inattention symptoms) and is designed to be similar to the ADHD-symptoms found in the "Diagnostic and Statistical Manual of Mental Disorders" (American Psychiatric Association, 2000). It has shown good psychometric properties (DuPaul et al., 1998). In Norway, there is a lack of official norms (Kornør & Bøe, 2011), but in Denmark, it has been standardized on approximately 600 children (Poulsen, Jørgensen, Dalsgaard, & Bilenberg, 2009). Both norms from the United States and from Denmark were used, with very similar results. A study from the United States has also supported the reliability and validity of ADHD RS-IV in PKU (Wyrwich et al., 2015).

The Norwegian version of BRIEF has shown good psychometric properties (Sørensen & Hysing, 2014). The parent version of BRIEF consists of 86 items that measure metacognition (Initiate, Plan/Organize, Working Memory, Organization of Materials and Monitor) and Behavioral Regulation (Emotional Control, Shift, Inhibit). Responses are given on a Likert scale indicating if the behavior of a child is "Never a problem," "Sometimes a problem," or "Often a problem."

2.3 | Enzyme purification and activity assays

Human TH isoform 1 and human TPH2 were expressed in *Escherichia coli* (BL21) and purified to homogeneity as described in Szigetvari et al. (2019) and Winge et al. (2008), respectively. Enzyme activities were measured in standard reaction mixtures (Szigetvari et al., 2019) at 37°C and were stopped after 5 min. The specific activity of TH was assayed at tyrosine concentrations ranging from 4 to 1,400 μM, while the concentration of tetrahydrobiopterin (BH_4) was kept constant at its estimated physiological concentration, 50 μM (Fossbakk, Kleppe, Knappskog, Martinez, & Haavik, 2014). TPH2 activity was measured at a substrate (tryptophan) concentration of either 20 or 60 μM, in the presence of 0–1,000 μM tyrosine. Formation of the reaction products L-DOPA and L-5-hydroxytryptophan, respectively, were detected via their native fluorescence, using high-performance liquid chromatography with fluorometric detection (Haavik & Flatmark, 1980). TH kinetic values were fitted by nonlinear regression analysis using the Michalies–Menten equation with substrate inhibition (Equation 1) in Graph-Pad Prism 7.0, where v is the rate of the reaction, S is the concentration of substrate, V_{max} the maximal rate, K_m the half saturation constant, and K_{si} is the substrate inhibition constant.

$$v = \frac{V_{max} S}{K_m + S\left(1 + \frac{S}{K_{si}}\right)} \quad (1)$$

2.4 | Statistical analyses

IBM SPSS Statistics 24 was used to perform the statistical analyses. The relationship between ADHD-related symptoms and levels of tyrosine was investigated using Pearson product–moment correlation coefficient. To check for normality, linearity, and homoscedasticity, preliminary analyses were performed, and nonparametric statistics (Spearman, 1904) were used when assumptions were violated. Pearson product–moment correlation analyses between variables from BRIEF and ADHD RS-IV were also performed (only when significantly correlated with tyrosine).

The relationship between inattentive symptoms and mean levels of tyrosine in 2017 was explored while controlling for age (months) at diagnosis. Preliminary analysis showed a violation to the assumption of linearity, therefore non-parametric partial correlation was chosen. A Spearman bivariate correlation was performed for all variables and the Spearman rank correlation coefficients was added into a new file. The row type from the Spearman (RHO) was converted to a Pearson product–moment correlation. Partial correlation was then performed using the newly created correlation coefficients. As we expected high

levels of tyrosine to be related to high levels of ADHD-related symptoms, one-tailed tests were used in all correlation analyses, except when performing Pearson product–moment correlation between the working memory subscale from BRIEF and the inattention scale from ADHD RS-IV.

3 | RESULTS

Mean plasma level of tyrosine from 2009 to 2016 was 477 μmol/L (range 358–644 μmol/L) and 623 μmol/L in 2017 (range 349–831 μmol/L). Mean age was 13.1 years (range 7–17) and mean age at diagnosis was 13 months (range 0–30). See Table 1 for individual characterization of participants.

All T-scores were around 50 or lower (50 = mean), both on the ADHD RS-IV (norms from Denmark and United States) and on BRIEF (Tables 2 and 3).

We observed a significant positive correlation between inattention symptoms on ADHD RS-IV and mean tyrosine level the last 8 years (r = .707, p = .025) and in 2017 (r = .780, p = .011; Figure 3a, b). The working memory index on BRIEF was significantly correlated with levels of tyrosine the last 8 years (r = .659, p = .038) (Figure 4), but not with mean level in 2017 (r = .593, p = .061). T-scores for inattention and working memory were also significantly correlated (r = .829, p = .011, two-tailed).

A strong positive correlation between levels of tyrosine in 2017 and symptoms of inattention (r = .839, p = .009) was found after controlling for age (months) at diagnosis.

TABLE 1 Characteristics of patients with tyrosinemia Type 1

Patient	Age	Months at diagnosis	Mean tyrosine at 8 years (μmol/L)	Mean tyrosine, 2017 (μmol/L)
1	13–18	>12	455 ± 93	609 ± 174
2	13–18	>12	421 ± 121	684 ± 19
3	13–18	<12	393 ± 102	627 ± 18
4	13–18	>12	634 ± 114	787 ± 85
5	13–18	>12	644 ± 182	831 ± 144
6	6–12	<12	490 ± 71	652 ± 77
7	6–12	<12	417 ± 145	445 ± 93
8	6–12	>12	358 ± 82	349 ± 204

TABLE 2 ADHD RS-IV, results according to United States and Danish norms (DN; N = 8)

	Minimum	Maximum	Mean	*SD*
Inattention	38	60	50.1	6.7
Inattention DN	37	61	51.3	7.8
Hyperactivity	40	68	49.9	8.7
Hyperactivity DN	38	67	50	9.4
Total	38	61	49.9	7.1
Total DN	36	60	50.5	8.1

TABLE 3 BRIEF T-scores, subscales, and indexes (N = 8)

	Minimum	Maximum	Mean	*SD*
Inhibition	36	60	47.0	8.9
Shifting	40	61	47.0	7.7
Emotional control	41	68	48.9	9.7
Initiate	39	63	47.4	7.1
Working memory	38	63	52.1	8.0
Plan/organize	39	63	51.1	7.8
Organization of materials	40	58	51.0	5.8
Monitor	32	49	43.1	5.7
Behavioral regulation index	40	62	47.5	8.9
Metacognition index	37	54	48.9	5.4
Global executive index	38	57	48.4	6.4

Abbreviation: BRIEF, behavior rating inventory of executive functioning.

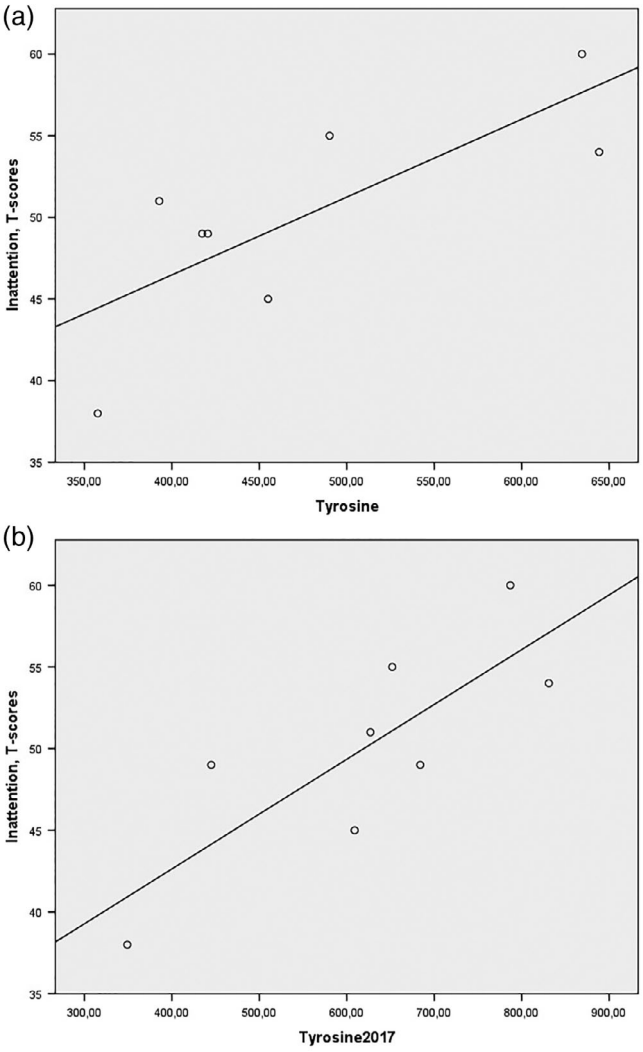

FIGURE 3 (a) Relation between mean levels of tyrosine (2009–2016) and levels of inattention on ADHD RS-IV. (b) Relation between level of tyrosine in 2017 and inattention on ADHD RS-IV

3.1 | Effect of pathological tyrosine levels on TH and TPH2 enzyme functions

We measured the activity of purified recombinant human TH isoform 1 (TH) at tyrosine concentrations spanning the range of normal plasma (19–119 μM, green area Figure 5a) (Gregory, Sovetts, Clow, & Scriver, 1986; Shih, 2003) and that reported for nitisinone-treated HT-1 patients (mean levels at or above 349 μM, red area Figure 5a). TH activity was measured at intracellular relevant physiological conditions, including BH_4 levels corresponding to the estimated intracellular concentration (Fossbakk et al., 2014). Maximal TH activity was observed at around 30 μM tyrosine. Substrate inhibition of TH was obvious even at physiological tyrosine levels. Comparing the enzyme velocity at median normal plasma tyrosine levels to that of median plasma level of nitisinone treated HT-1 patients, showed an 81% reduction—alternatively, a 71–85% decrease within the 349–831 μM tyrosine range reported from HT-1 patients under treatment (Figure 5a), suggesting that catecholamine synthesis may be compromised under such conditions (Table 4).

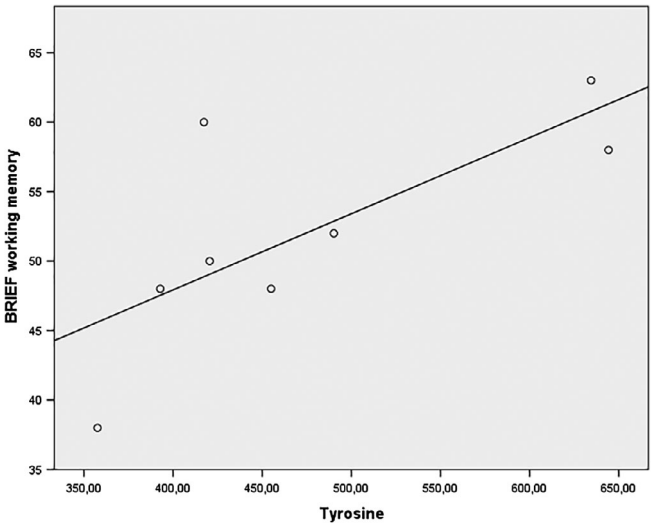

FIGURE 4 Relation between mean plasma tyrosine concentrations (2009–2016) and working memory index from BRIEF. BRIEF, behavior rating inventory of executive functioning

TABLE 4 Specific activity of human TH1 measured under physiological conditions

V_{max} (nmol min^{-1} mg^{-1})	K_m (μM)	K_{si} (μM)	R^2
2,822 ± 511.1	30.61 ± 7.76	28.91 ± 7.01	0.971

Note: V_{max}, K_m (tyrosine concentration at half maximal rate), and K_{si} (substrate inhibition constant for tyrosine) values shown here represent the best-fit values within 95% confidence interval ± *SD*. The substrate inhibition of TH is obvious present even at physiological tyrosine levels. There is an apparent overlap between K_m and K_{si} values. The explanation for this is that the standard substrate inhibition kinetic equation employed here cannot factor in the consequences of the orderly nature of substrate and cofactor binding by TH during the catalytic reaction, as it can only do the fitting for a single substrate variable.

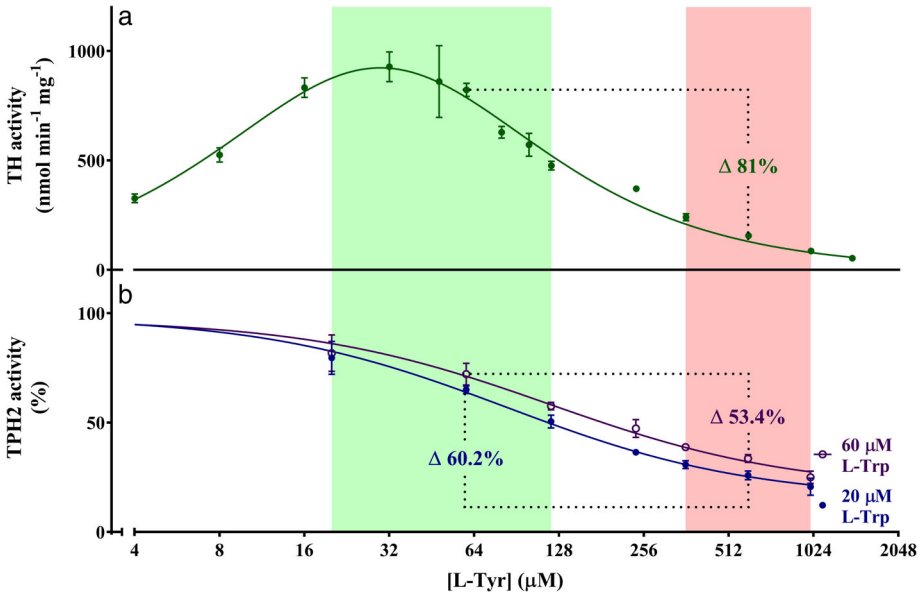

FIGURE 5 Effect of tyrosine on TH and TPH2 activity. The activity of TH and TPH2 was assessed in the presence of different concentrations of tyrosine ([L-Tyr], note logarithmic scale on *x*-axis). For assay conditions and procedures, we refer to Section 2. The green shaded area represents the normal plasma levels of tyrosine (19–119 μM) and the red shaded area plasma tyrosine concentrations reported for nitisinone treated HT-1 patients. (a) Shows TH activity as a function of increasing amounts of tyrosine. TH activity increases toward about 30 μM Tyr, but decreases above this level due to substrate inhibition. (b) TPH2 activity (%, 100% without tyrosine present) measured at 20 μM (●) or 60 μM (○) tryptophan and with increasing amounts of tyrosine present in the assay. TH, tyrosine hydroxylase; TPH2, tryptophan hydroxylase 2

A decrease in serotonin levels has also been reported in HT-1 (Thimm et al., 2011). Consequently, we examined the effects of increasing tyrosine levels on the activity of human TPH2, the rate-limiting enzyme of brain serotonin synthesis (Figure 5b). We observed 53–60% reduction in TPH2 activity going from median normal plasma tyrosine levels to that of median plasma levels of nitisinone-treated HT-1 patients. The decrease in activity was more prominent with lower tryptophan concentrations (from 46–59% to 53–64% reduction in catalytic activity in the tyrosine range of 349–831 μM, after tryptophan was reduced threefold), suggesting a competitive inhibition by tyrosine.

4 | DISCUSSION

4.1 | Summary of findings

In this study, we observed strong correlations between symptoms of inattention and both recent and long-term plasma levels of tyrosine in HT-1 patients receiving nitisinone medication. We also found a negative correlation between working memory and tyrosine levels. This was expected, as the working memory scale from BRIEF and the inattention scale from ASRS RS-IV were highly correlated, as also shown in other studies, for example, in patients with PKU (Wyrwich et al., 2015).

It has been speculated whether the high plasma tyrosine levels found in HT-1 patients on nitisinone treatment would increase dopamine synthesis in the brain (van Ginkel et al., 2017). However, our biochemial studies on human TH indicated that at such extreme levels of tyrosine, the activity of the enzyme may be reduced by substrate inhibition. Thus, we hypothesize that the inhibition of TH activity also reduces the levels of dopamine and norepinephrine in the brain.

4.2 | Shared phenotypes between ADHD, phenylketonuria, and HT-1?

ADHD is suggested to be associated with decreased activity of dopamine in prefrontal cortex (Volkow et al., 2009) and is found to be 2.5 times more prevalent in PKU than in the general population, with high level of dimensional symptoms also in patients not fulfilling the criteria for diagnosis (Antshel & Waisbren, 2003b). Central stimulants, such as methylphenidate, the most common pharmacological treatment for ADHD, inhibits the synaptic dopamine and noradrenaline transporters, thereby increasing the concentration of these catecholamines in the synapses. This reduces core symptoms in a majority of children with ADHD (Swanson et al., 2001; Wilens & Biederman, 1992), improves motoric functioning (Stray, Ellertsen, & Stray, 2010) and reduces negative social behavior (Gadow, Nolan, Sprafkin, & Sverd, 1995; Gillberg et al., 1997; Hinshaw, Henker, Whalen, Erhardt, & Dunnington, 1989). Interestingly, the neurodevelopmental problems seen in diet-treated PKU and ADHD are very similar. In PKU, problems with executive functioning (Huijbregts, Gassio, & Campistol, 2013) and lower social skills (Jahja et al., 2016) are described, in addition to impaired motor function, working memory and attention (summarized in a meta-analysis by Stevenson and McNaughton (2013)). Stevenson and McNaughton have examined whether PKU and ADHD give common phenotypes and postulated that reduced dopamine activity in the prefrontal cortex may be responsible for cognitive impairments in PKU, similar to the etiology proposed in ADHD by Solanto (2002).

An impaired serotonin homeostasis has also been reported in treated HT-1 (Thimm et al., 2011); this was suggested to stem from impeded transport of tryptophan across the BBB. Similarly to the disruption of TPH activity in PKU, attributed to high phenylalanine levels, an analogous inhibitory effect by tyrosine was speculated. Here, we suggest that the reduced serotonin synthesis and cognitive symptoms in HT-1 may be manifestations of the cumulative effects of decreased brain tryptophan availability, as well as inhibition of TPH2 by the competing tyrosine.

Our study provides more insight into the hitherto unexplained similarities between cognitive difficulties found in treated HT-1, ADHD, tyrosinemia Type 2 and 3 as well as PKU. The strong correlation between recent tyrosine concentrations and levels of inattention, and the even more robust correlation when correcting for age at diagnosis, support the notion that transient concentration of tyrosine could be more relevant for levels of inattention than long-term concentrations, and that these effects are not directly attributed to nitisinone itself. Garcia, de la Parra, Arias, Arredondo, and Cabello (2017) reported a decline in IQ (mean of 16.8 IQ points) over minimum 2 years in patients receiving a diagnosis before 8 months of age (with first symptom appearing between the first and third months). Bendadi et al. (2014) also found a decline in IQ in a group with HT-1 who were 8 months or younger when receiving the diagnosis. Interestingly, in their study, the IQ and tyrosine levels were not correlated. Furthermore, the decline in IQ values reported by Garcia et al. was not found in children diagnosed between 8 and 47 months of age. In contrast to prior beliefs, early brain damage has been related to decline in IQ over the course of development, especially with relatively small lesions. This was found in a study of children with unilateral brain injury (mainly with pre- or perinatal infarctions), but decline in IQ over time has also been found in children with Trisomy 21 (Carr, 1988) and Fragile X syndrome (Hagerman et al., 1989). Similarly, it may be that early onset of HT-1 impacts the vulnerable infant brain more or differently, compared to those presenting with symptoms later. While it has been difficult to demonstrate a clear correlation between IQ-scores and metabolic control, a study by Pohorecka et al. (2012) found that attention difficulties were related to fluctuating levels of tyrosine (in a group with IQ similar to the population mean) and the study by Walker et al. showed higher levels of tyrosine in patients performing poorly on neuropsychological assessment (Walker et al., 2018). Performance on a load-dependent working memory task also decreased with higher levels of orally administered tyrosine in adults (van de Rest, Bloemendaal, de Heus, & Aarts, 2017). This indicates that tyrosine levels may be more strongly connected to the other cognitive problems described in this group than to IQ values. However, in the study by Pohorecka et al., an association between verbal IQ and inattention was found, and a meta-analysis has shown a

nine point lower IQ in patients with ADHD than in a control group of healthy siblings (Frazier, Demaree, & Youngstrom, 2004). Despite this, these findings do not explain why mean IQ of patients with HT-1 in some studies are found to be as low as 71 (Bendadi et al., 2014). In comparison, in a study of children with early treated PKU, the mean IQ was 91 (Griffiths, Demellweek, Fay, Robinson, & Davidson, 2000); only nine points below the norms. If HT-1, ADHD and PKU share biological mechanisms and a common phenotype regarding ADHD-related problems, a similar influence on IQ from the ADHD-related symptoms could be expected in HT-1 as in PKU and ADHD. Therefore, the very low IQ-levels found in some studies of HT-1 (and not in others) possibly point to partly different trajectories for reduced intellectual functioning in HT-1 and ADHD/PKU, and subgroups with partly different etiology behind the cognitive difficulties in HT-1. Accordingly, medication aiming to increase brain levels of catecholamines, such as inhibitors of dopamine and norepinephrine transporters, may be useful in the treatment of ADHD-related difficulties in HT-1, as also demonstrated for some of the children in this study (data not shown). Research on biological mechanisms and possible treatments for ADHD-related symptoms in HT-1 could provide more refined venues for medical intervention through dietary control and compensatory treatment.

4.3 | Executive functioning and HT-1

On the BRIEF scale, only working memory was correlated with levels of tyrosine, and this subscale was also highly correlated with the inattention subscale from ADHD RS-IV. Although that some researchers consider problems with executive function as core deficits in ADHD, others have argued that although such difficulties are important in ADHD, they are neither sufficient nor necessary in all cases of ADHD (Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005).

4.4 | Decline in cognitive functioning over time?

In all but one participant, mean levels of tyrosine in 2017 were higher than those from the 2009 to 2016 period. This may be related to the fact that it has been discussed if recommended levels of P-tyrosine <600 μmol should be suggested (instead of a level <500 μmol). In 2017, this was also included in guidelines (Chinsky et al., 2017). On the other hand, if this finding could be generalized to patients with HT-1, a possible decline in cognitive functions could be attributed to the increased tyrosine levels. Both the decline in IQ documented in other studies, and the possible decline in other cognitive functions point to the importance of considering repeated neurocognitive assessments as standard follow-up procedures for this group.

4.5 | Diagnostic overshadowing?

Recently, the term "diagnostic overshadowing" was used to describe the underdiagnoses of comorbid psychiatric conditions in children with severe neurological diseases (Hendriksen, Peijnenborgh, Aldenkamp, & Vles, 2015). The authors suggested that more obvious and important somatic features may overshadow a comorbid ADHD. In Norway HT-1 was not included in the mandatory screening program for newborns until 2012. Thus, few of the participants in this study were recognized before presenting with serious symptoms. One hypothesis may be that a delayed diagnosis contributes to diagnostic overshadowing, as several of the parents probably have gone through considerable strain before the diagnosis was settled. If the main focus has been on survival of the child, diffuse symptoms as inattentiveness may have been overlooked or neglected. If we assume that this possible bias is constant in the group, the relative differences between reported problems might still be valid. Paradoxically, underdiagnosing or overlooking impairing symptoms of ADHD may be especially harmful to individuals who are dependent on strict treatment-regimes for their medical condition, because of the unstructured lifestyle that sometimes accompanies untreated ADHD. Therefore, early identification of ADHD and treatment in medical conditions like HT-1 is important from a lifetime-perspective, as this may also improve prognosis for the medical condition itself. This has been demonstrated in adolescents with type 1 diabetes, where poor metabolic control was found in patients with undiagnosed ADHD (Nylander, Lindstrom, Khalifa, & Fernell, 2018).

5 | CONCLUSION

NMDs, such as HT-1, constitute a large group of conditions that are often containable with early clinical intervention, but still present lifelong difficulties and high societal costs. The long-term consequences of treatments, despite their beneficial short-term effects should also be investigated. This study suggests that there may be similar biological mechanisms behind the cognitive difficulties seen in PKU, ADHD and HT-1. In clinical settings, the impaired dopamine synthesis due to substrate inhibition in treated HT-1 may be compensated for by standard ADHD medication, such as methylphenidate or amphetamine. Similarly, the reduced serotonin synthesis may be counteracted by tryptophan supplementation. Thorough assessment of ADHD and monitoring of side-effects in accordance with clinical guidelines is required for treatment with stimulants. In future studies, comparisons of PKU and HT-1, in addition to other metabolic disorders influencing similar biological pathways, will hopefully provide more insights into possible shared pathophysiological mechanisms and how these affect their treatment.

5.1 | Strengths and limitations

The main limitation of this study is the small number of participants, which reflects the low prevalence of HT-1. Despite the limited sample, strong correlations were obtained. Still, replication studies and studies with larger samples are needed.

The present results only show a correlation between inattentiveness and tyrosine levels, whereas causality may go both ways, as inattentiveness also could lead to difficulties in managing diet (similar to the Nylander et al. study of diabetes). However, if this was the case in

this study, more general difficulties with executive functioning should be suspected in patients with the highest levels of tyrosine.

We only used parent versions of questionnaires to obtain data, and ideally information from teachers should also have been available. In future studies, a complete assessment of ADHD in HT-1-patients would give a more accurate picture of the prevalence of ADHD in this group. A psychiatric diagnostic interview should also be part of such an assessment. However, using dimensional data, as in this study, may give important information about relationships between symptoms of ADHD and biological measures, in addition to capturing the dimensional nature of this psychiatric condition. Ideally, in future studies both categorical and dimensional data should therefore be obtained. It also may be of interest to collect experimental neuropsychological data, especially because of the possible "diagnostic overshadowing" and underreporting of symptoms. However, questionnaires could be more valid than test results when assessing daily functioning and predicting job performance (Barkley & Murphy, 2010). In further studies, a combination of questionnaires and neuropsychological tests may be preferable, as they measure different aspects of neurocognitive functioning (Barkley & Murphy, 2010). IQ should also be measured to aquire more knowledge about the etiology behind the different aspects of cognitive functioning in HT-1.

We did not measure tyrosine concentrations, or rates of monoamine synthesis directly in nerve terminal in relevant brain tissues in the affected patients. Although a strong correlation has previously been reported between plasma levels of tyrosine and its concentration in cerebrospinal fluid in HT-1 patients (Thimm et al., 2011), a recent magnetic resonance spectroscopy study on PKU patients indicated a large concentration gradient between blood and brain (posterior cingulate gyrus and perventricular white matter) levels of phenylalanine and tyrosine (Waisbren et al., 2017). Still, the effective subcellular concentration of amino acids in relevant compartments of monoaminergic neurons in these patients is not known. Furthermore, while this study focused on effects on monoamine synthesis, nitisinone treated HT-1 patients may have other biochemical disturbances that also may affect brain functions.

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CONFLICT OF INTEREST

J.H. has served as a speaker for Eli-Lilly, HB Pharma, and Shire. The other authors declare no conflicts of interest.

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